PCt-RFLP MITOCHONDRIAL ANALYSIS: RAPID DISCRIMINATION OF ACIPENSER BAERII AND TRISOPTERUS MINUTUS MINUTUS EGGS

Abstract - Species identification is a key problem throughout the life cycle of fishes: from eggs and larvae to adults in ecosystem and fisheries research and control, as well as processed fish products labelling in consumer. Amplification of a region of the mitochondrial genome, the cytochrome b, using the polymerase chain reaction (PCR) permits species discrimination. The obtained long PCR-products cut with different restriction endonucleases resulting in species-specific restriction fragment length polymorphisms (RFLP) allow to discriminate between different types of eggs species. The following analysis of mitochondrial DNA is suitable with only one enzymes, HpaII, to differentiate and control the labeling of the caviar, Acipenser baerii and the caviar substitution specie, Trisopterus minutus minatus

Key-words: caviar, mitochondrial DNA, PCR, RFLP.

Introduction - Caviar, as known, is the most commercially valuable species, among species distributed worldwide and they are among the most endangered freshwater fishes. In order to fulfil the obligation to control international caviar trade, a more effective and accurate species identification system is essential. Historically, sturgeon identification in the trade was based on a comparison of the size and color of eggs (Chen et al., 1996). Normally, sturgeons spawn several times during their life, and the size of eggs depends on the species and the age of females. Species identification methods based on the analyses of specific proteins is not always applicable. Alternatively, nucleotide sequencing and restriction fragment length polymorphism (RFLP) procedures are useful (Guerriero et al., 2010). The objectives of the present study were to confirm the egg fish species by PCR sequencing and to detect the restriction enzymes to use with PCR-RFLP method for the sturgeons Trisopterus minutus minutus and the caviar, Acipenser baerii.

Materials and methods - Samples, listed as Trisopterus minutus minutus and Acipenser baerii were bought in different market sites (Italy and Egypt). The genomic DNA was extracted from 100 mg of eggs. PCR amplification of the 389 bp cytochrome b rRNA gene fragment was performed as previously reported (Guerriero et al., 2010), in ten aliquots of each samples. Amplified DNA was desalted with Microcon 100 spin columns and sequenced. The obtained sequence were compared for control with Fasta sequences data for cytb tRNA belonging to those species examined. Virtual restriction maps of cytb rRNA gene sequences were obtained using NEB cutter. PCR products derived from amplification of cytb rRNA gene were subjected to restriction digestion without previous purification using Hpa II restriction enzyme (M-Medical srl, Milan) and reaction mixtures specified by the manufacturer.

Results - In all samples listed as Trisopterus minutus minutus and Acipenser baerii we obtained a fragment of ~389 bp length from the cytb mt-rRNA gene (data not
shown). The sequences of the amplicons obtained and analysed by comparing them with those reported in the database by a FASTA analysis confirm the attribution of species reported in the label. Furthermore, the cleaved fragments obtained from PCR amplicons of the ~389bp cytb mt-rRNA fragment with Hpa II endonuclease resulted in agreement with those expected by theoretical analysis of our obtained sequences (Tab. 1). The fragments were visualized by electrophoresis in 2% agarose gel and provide evidence of two different restriction patterns for the species examined (Fig. 1).

Tab. 1 - Theoretical restriction fragment length polymorphism of the ~389bp cytb mt-rRNA fragment in *Trisopterus minutus minutus* and *Acipenser baerii*.

<table>
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<tr>
<th>Species</th>
<th><em>HpaII</em> Position/Fragments length (bp)</th>
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<tbody>
<tr>
<td><em>Trisopterus minutus minutus</em></td>
<td>13/13; 377</td>
</tr>
<tr>
<td><em>Acipenser baerii</em></td>
<td>211/190; 211</td>
</tr>
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Conclusions - The increasing sequence information, acquired in DNA databases such as NCBI, EMBL and DDBJ culminated in an advance to include molecular species identification into the existing CITES regulations. Actually, our study show the RFLP analysis obtained by only one restriction enzyme, *HpaII* that allows an unequivocal discrimination between the sturgeon specie, *Acipenser baerii* and the substitution caviar *Trisopterus minutus minutus*. Taking into consideration the great commercial value of these species, this method showed a rapid way for exposing commercial frauds, like mislabelling of caviar lots, to avoid commercial frauds on the fish market and to identify juvenile use in restocking programs.

References
